ASSIGNMENT-1

1) The observations made by Charles Darwin during his visit were:

i) In each island, there were unique species of birds, tortoises and other creatures and no two islands had the same type of species. Species similar to them were found in the neighbouring islands. Darwin further observed, that in a given population of a species, say humans, there are individuals with different features (height, colour of hair/eyes). These differences within a species (also called variations) are heritable.

ii) Though the species were different in the various islands (based on environment e.g. islands), species could adapt to the environments of other islands. For eg. islands having vegetation at a much lower quantity, the neck of the tortoises were smaller. In islands with high level of vegetation, the tortoises had a long neck. Therefore, the species could adapt to the changing environment.

iii) As populations grow in size, the resources become limited. This leads to a competition among the individuals for the resource, leading to "survival of the fittest". Individual with variations that allow them to adapt to the environment are most likely to survive and reproduce and also pass the favourable characters to the next generation

In short :

- Individuals in a population exhibit variable traits : variations
- Many traits are inheritable
- Species adapt to their environment
- Individuals with traits that allow them to adapt to the environment are most likely to survive and reproduce.

2) Microbiome is the genetic material of all microbes in the human body (bacteria, fungi, protozoa, virus)

The bacteria in our microbiome, helps in digestion of our food, protects against other bacteria that cause diseases. They also help in producing vitamins (B, B12), thiamine, riboflavin and also vitamin K needed for blood coagulation

Therefore, we can say that the microbiome is essential for human development, immunity and nutrition.

3) Given mRNA sequence: 5' AUGGUGGCCUAUCAUUAGGGGCUU 3'

i) Amino acid sequence of the polypeptide encoded by the above mRNA sequence is:

Met Val Ala Tyr His "STOP". So, we ignore the stop codon

ii) and write — Met Val Ala Tyr His Ans:

On making the apt change, the new mRNA sequence is:

5' AUGGUGGCCUA**A**CAUUAGGGGCUU 3'

Upon translation we get: Met Val Ala Ans: (Ignoring "STOP" codon).

iii) On making the required change, the nfo mRNA sequence is:

5' AUG**C**GUGGCCUAUCAUUAGGGGCUU 3'

Upon translation we get: Met Arg Gly Leu Ser Leu Gly Ala Ans:

Note: Above answers based on codon usage table of E.coli

4) Any virus has an envelope glycoprotein that helps in initiating the entry of the virus into the host cells. It binds to cell surface receptors which ultimately leads to membrane fusion and delivery of the genome into the cytoplasm. SARS-CoV 2 is also the same and this glycoprotein form

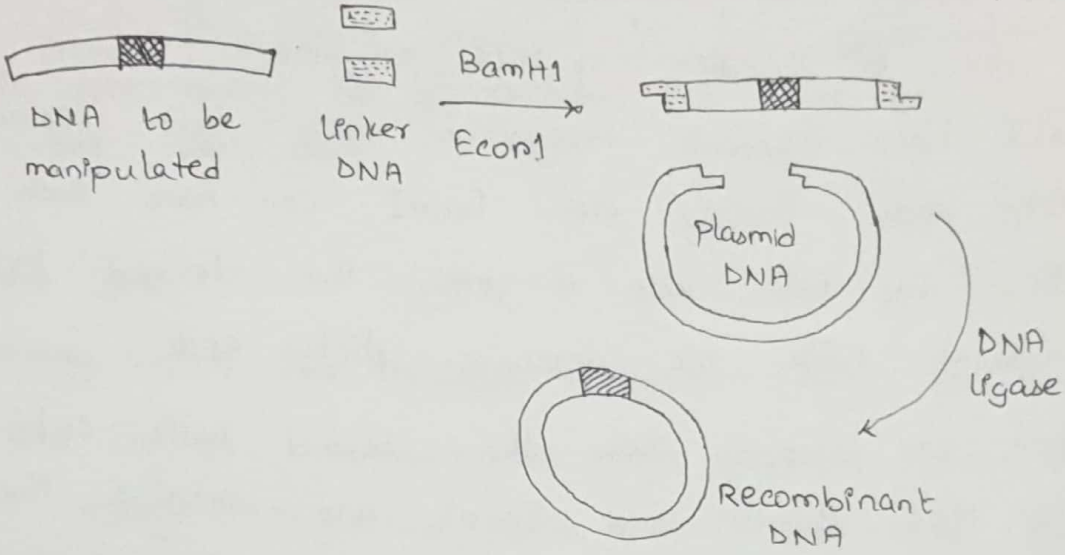
the characteristic corona of large, distinctive spikes(S) in the envelope. These small surface projections form the periphery of the SARS-CoV2 virus.

In a research on SARS-CoV2 S glycoprotein by the US National Institute of Health, full-length S glycoprotein cDNAs were constructed and certain "primers" were used for amplification and overlapping PCR cloning of the S glycoproteins. As per the research this full-length S-gene was cut with BamH1 and XbaI. ~~and digested~~ We know that BamH1 identifies the sequence — GGATCC and makes a cut at the site between two Gs. So from here we understand that we will find the above sequence in the glycoprotein of the virus. So, after cutting the glycoprotein, the virus will not be able to enter the host cell.

5) There are multiple ways to solve this problem:

i) We can use linker molecules for this problem. Linkers are short double stranded DNA molecules with recognition sites for RE.

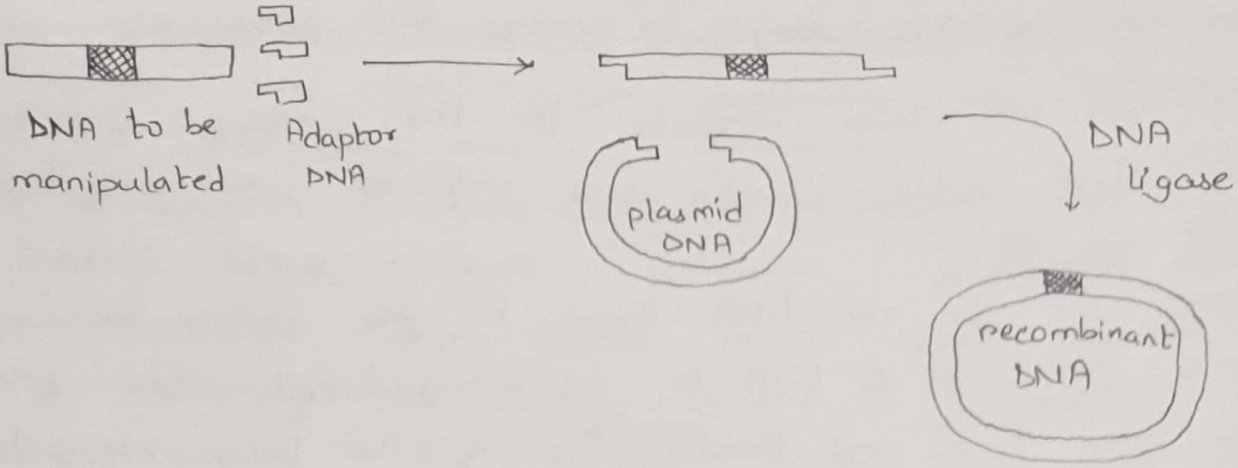
Here, we can use DNA ligase to join linkers on both the sides of DNA to be manipulated. Then we can have EcoRI and BamHI to cut the linker recognition sites. After that we can combine the plasmid DNA's cohesive ends and the same sticky ends from the linker molecules attached to the DNA to be manipulated.



ii) We can use adaptor molecules for this problem. Adaptors are chemically synthesized DNA molecules with pre-formed cohesive ends. They have one blunt end and another cohesive end.

Here we can create adaptor molecules with cohesive ends that are produced by EcoRI and BamHI. The blunt end has 5' phosphate group and the cohesive end is not phosphorylated to prevent self-ligation.

We can join the DNA to be manipulated and adaptor molecules. Then use DNA ligase to combine it with plasmid DNA(already cut by EcoRI and BamHI) at the cohesive ends to get the recombinant DNA.



iii) There can be possibly be a third way.

We try to experiment with other REs that leave the same sticky ends as EcoR1 and BamH1. For example, in the 6th Question of this assignment BamHI and BalII have different recognition sites but leave the sticky ends. Possibly even EcoRI can have such similar REs. This will help in getting the desired DNA fragment with the necessary sticky ends.

Then, we already have the plasmid DNA cut with the RES BamH1 and EcoR1 and contains the same sticky ends as the DNA fragment. We finally use DNA ligase to join the DNA fragments and plasmid DNA to get the recombinant DNA.

6) i) Both BamH I and Bal II have length of their RE sites as 6.

In a random DNA sequence, the 4 bases – A, T, G, C are present with equal probability

Since both have the same length of RE sites, both of them will occur once per $(4)^6$ times, or, the probability of the RE sites occurring is $\frac{1}{(4)^6}$.

ii) The advantage of having such a pair of REs is that there will always be a high probability of having restriction sites with desired sticky ends that flank our DNA fragment of interest.

For eg: BamHI, BgIII and Sau3A have different restriction recognition sites — BamHI has G↓GATCC; BgIII has A↓GATCT; Sau3A has ↓GATC but they have the same sticky end "GATC". So, this ensures that we will always find one or the other recognition sites flanking our DNA fragment. Then it can combine with the plasmid DNA (to form the recombinant DNA) with same sticky ends.

7) Comparing (molecular) cloning and PCR:

(i) Cloning replicates the DNA within the cell, whereas for PCR it can be done outside, in an "in vitro solution" without living cells. [PCR better]

(ii) Cloning means cutting and pasting sequences, but PCR amplifies DNA by coping an already existing sequences. [PCR better]

(iii) DNA cloned using "Cloning" method are correctly copied and fully functional. PCR however introduces errors in the sequence, leading to mutations. [Cloning better]